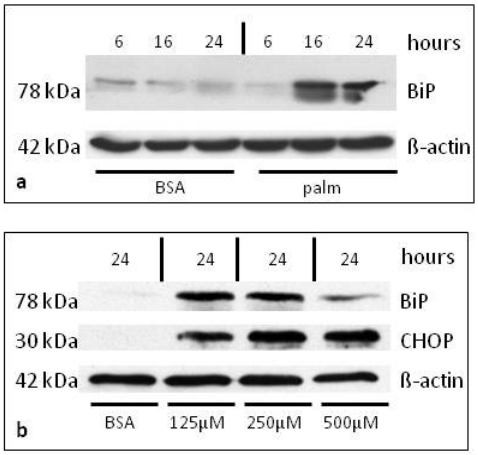
SUPPORTING MATERIAL

MATERIALS AND METHODS

Quantitative real-time PCR of renal biopsies - Human renal biopsy specimens were procured in an international multicenter study, the European Renal cDNA Bank-Kroener-Fresenius Biopsy bank (ERCB-KFB). Biopsies were obtained from patients after informed consent and with approval of the local ethics committees. Following renal biopsy, the tissue was transferred to RNase inhibitor and microdissected into glomerular and tubular fragments. Total RNA was isolated from micro-dissected glomeruli as described previously (1). Reverse transcription and real-time RT-PCR were performed as reported earlier (1). Pre-developed TaqMan reagents were used for human BiP (NM_005347.2), HYOU1 (NM_006389.2), CHOP (NM_004083.4), as well as the reference genes (Applied Biosystems). The expression of BiP, HYOU1 and CHOP was normalized to the mean of three reference genes, GAPDH, 18S rRNA, and synaptopodin. The mRNA expression was analyzed by standard curve quantification (4). For the real time RT-PCR data statistical analysis was performed using Kruskal-Wallis and Mann-Whitney U tests.

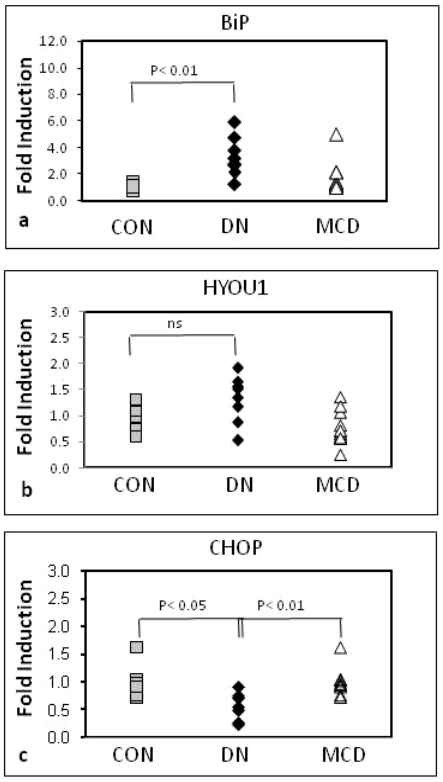
SUPPLEMENTAL RESULTS

SFig 1.: Dose and time-dependent induction of the ER-chaperons BiP and CHOP



A. Time-dependent upregulation of BiP protein levels by plamitic acid. b-actin serves as loading control. Representative results of 3 independent experiments. *B.* Upregulation of BiP and CHOP at 125µM, 250µM, and 500 µM palmitic acid. For the control condition (BSA) the BSA concentration was equivalent to cells exposed to 500 µM palmitic acid complexed to BSA. b-actin serves as loading control. Representative results of 2 independent experiments.

SFig 2.: Induction of UPR gene mRNA expression in glomeruli of patients with DN



(A-C) mRNA expression levels of (*A*) BiP, *(B)* HYOU, and (*C)* CHOP were quantified in microdissected glomeruli from controls (CON, n = 6), patients with established DN (DN, n=8) and patients with MCD (n=12). BiP (*A*) was upregulated and CHOP (*C*) was downregulated in patients with DN compared to control samples. *(B)* No significant changes were found for HYOU1. The changes between controls and MCD were not significant for all three genes. The graphs for BiP, HYOU1, and CHOP show expression ratios of each gene normalized to three reference genes (18S rRNA, hGAPDH, and synaptopodin).

Supplemental Table 1: Clinical data from living donors and patients with DN or MCD analyzed by real time RT-PCR

Biopsy Group	Number	Age [years]	Creatinine [mg/dl]	Proteinuria [g/day]	Hypertension	HbA1c (%)	RR syst.	RR diast.
Living donor								
Mean ±	6	46.2 ±	<1.1	<0.2	0/6	NA	NA	NA
SEM		13.8						
Diabetic Nephropathy								
Mean ±	8	60.4 ±	2.5 ±	4.8 ± 3.8	8/8	8.2 ± 2.6	157.9 ±	85.9 ±
SEM		8.4	1.8				11.2	4.0
Minimal Change Disease								
Mean ±	12	33.3 ±	1.1 ±	6.7 ± 4.5	4/12	NA	129.2 ±	75.3 ±
SEM		13.6	0.3				4.9	3.1

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